

MEGAKARYOCYTE AND PLATELET DEVELOPMENT

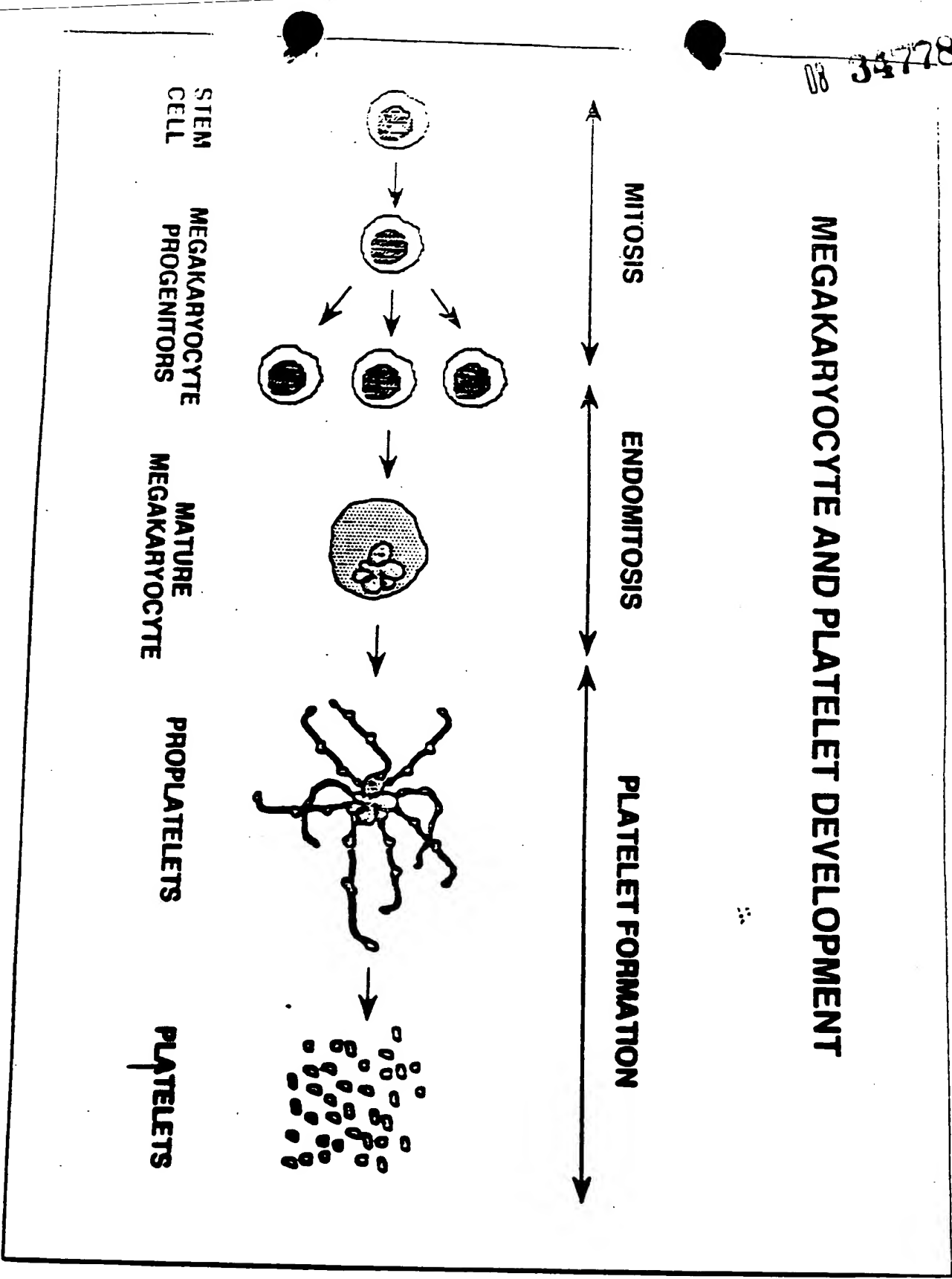


Figure 1

MPL-X completely blocks the ability of APK9 to induce megakaryocyte development

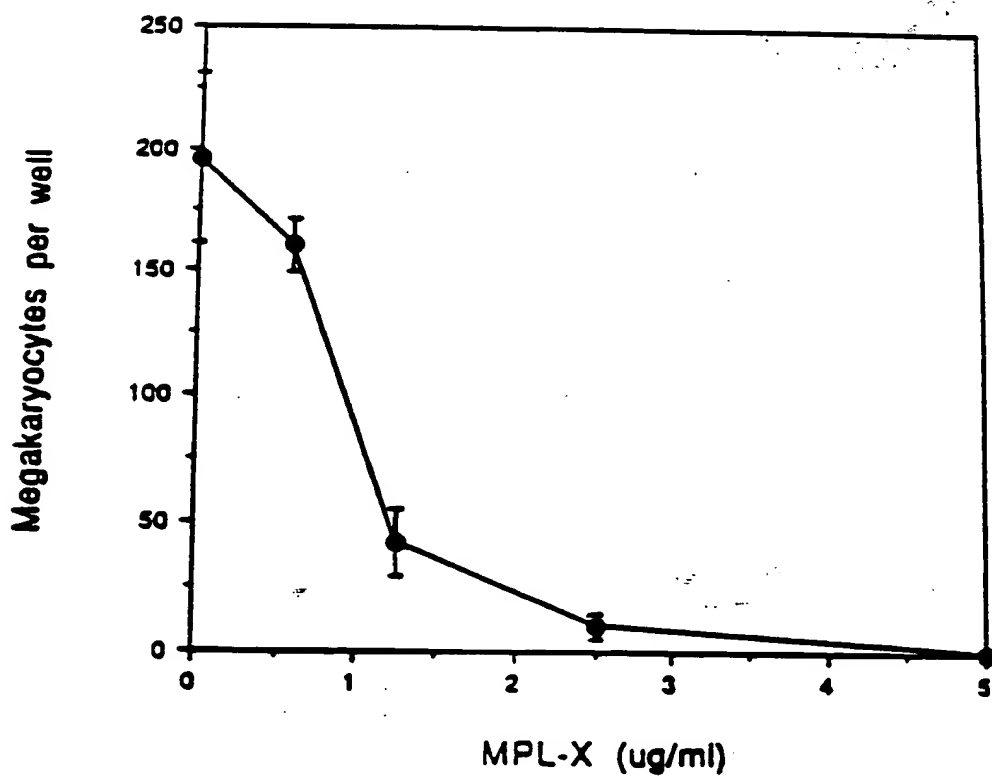


Figure 2

MPL Ligand Stimulates 1A6.1 cell growth

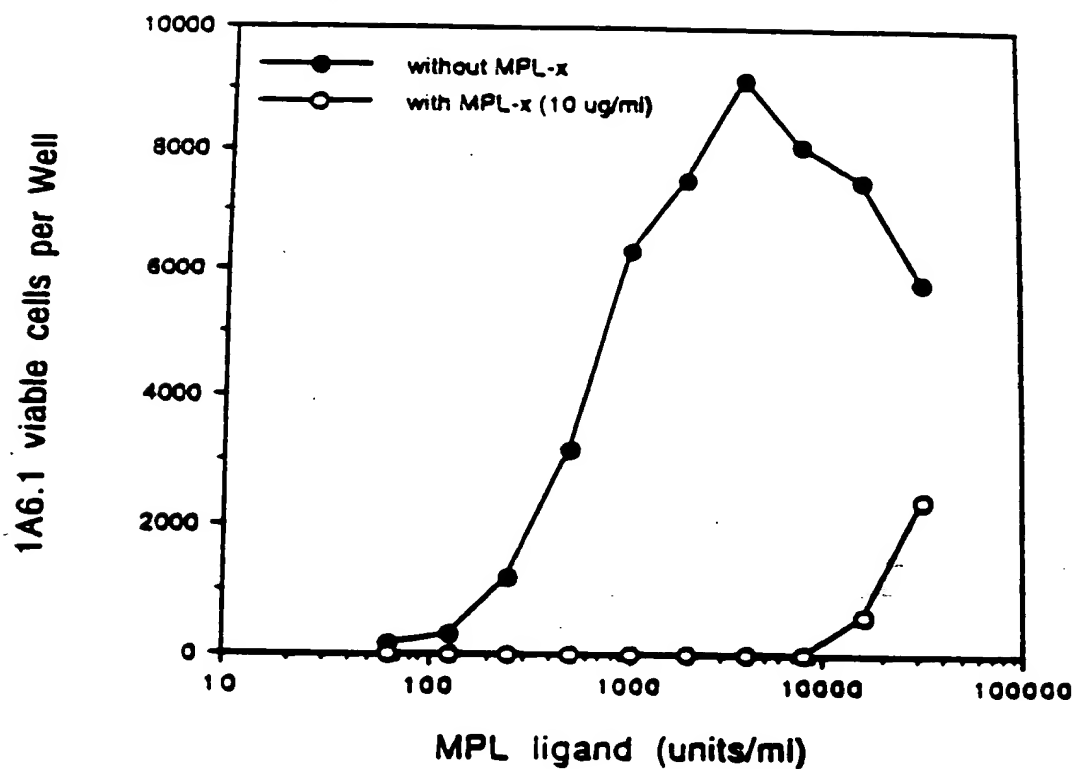


Figure 3

Purification of Mpl ligand

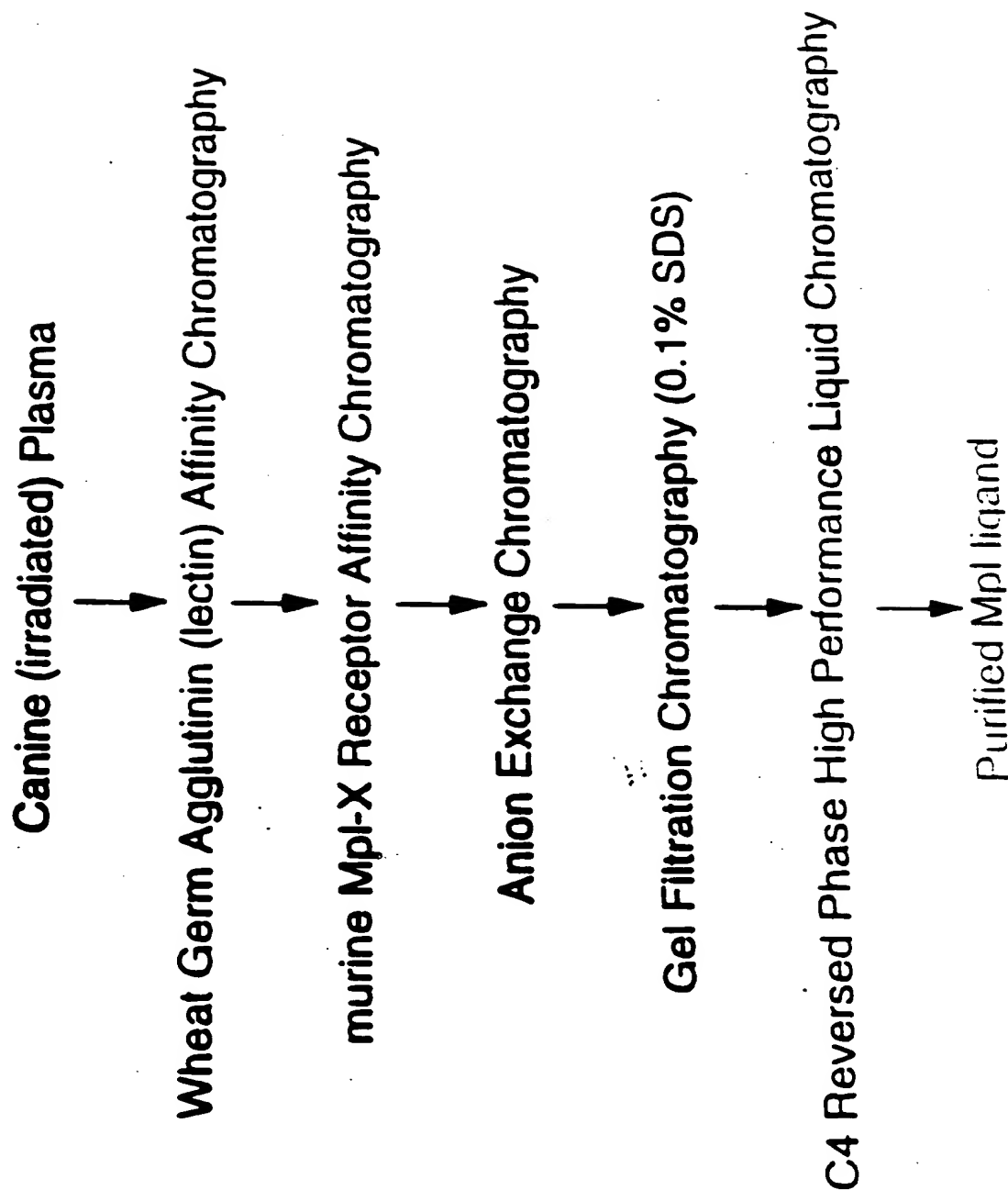
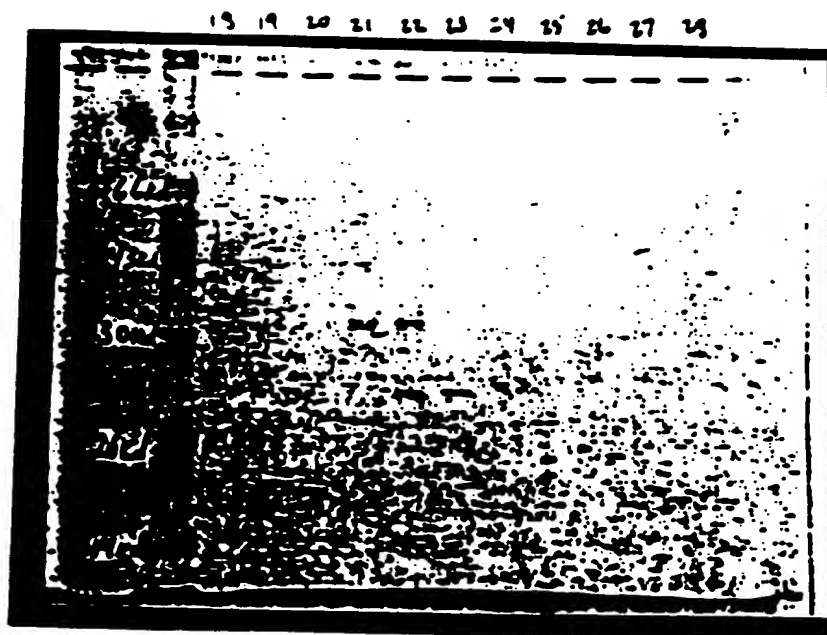
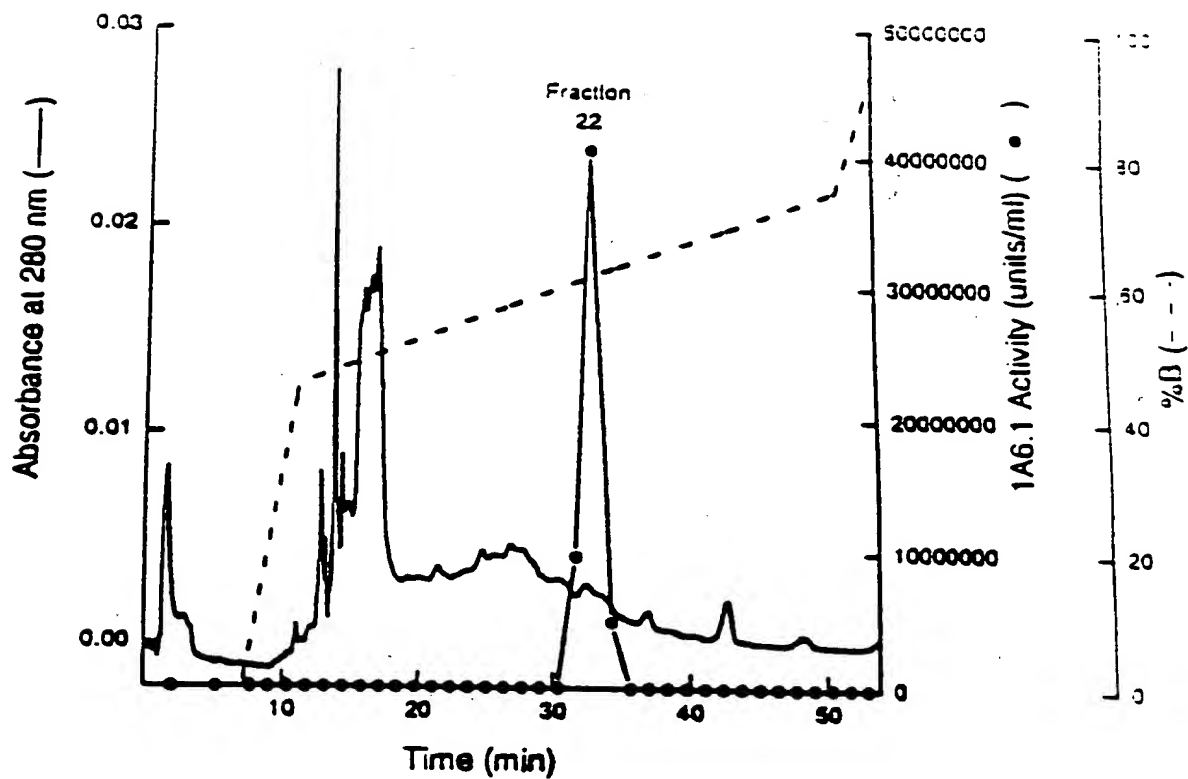


Figure 4

C4 RP-HPLC Purification of Mbl Ligand
 Sample = Superdex 200 Fraction 42

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SDS-PAGE 14% NONREDUCING

Figure 5

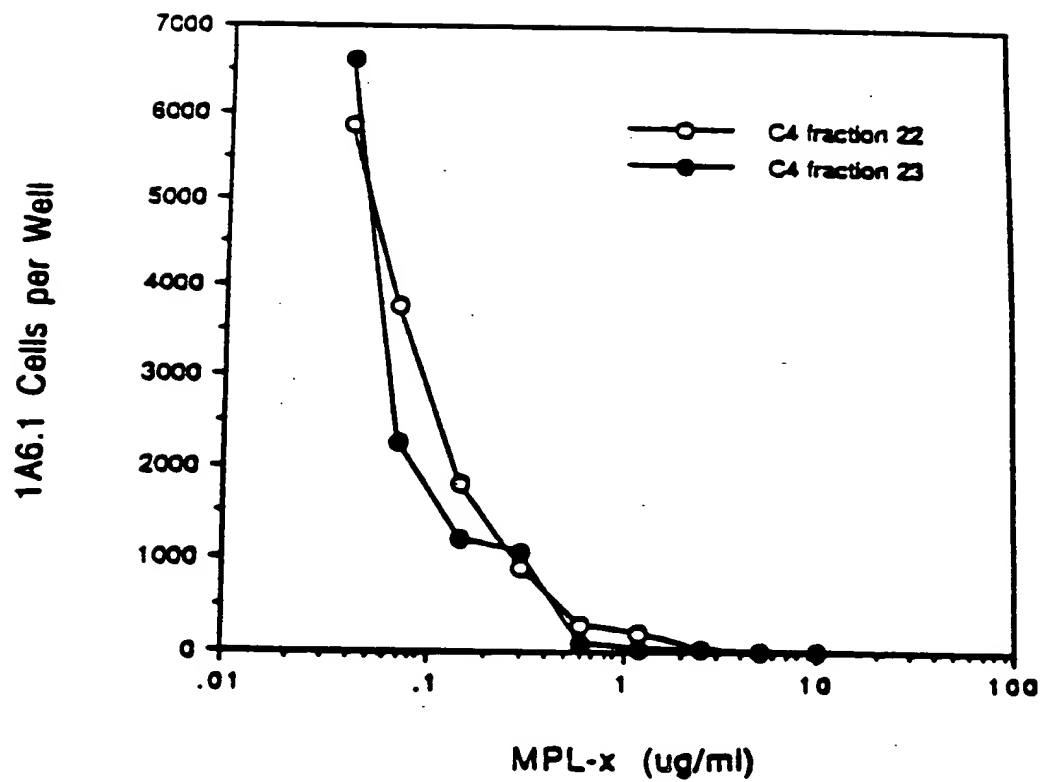


Figure 6

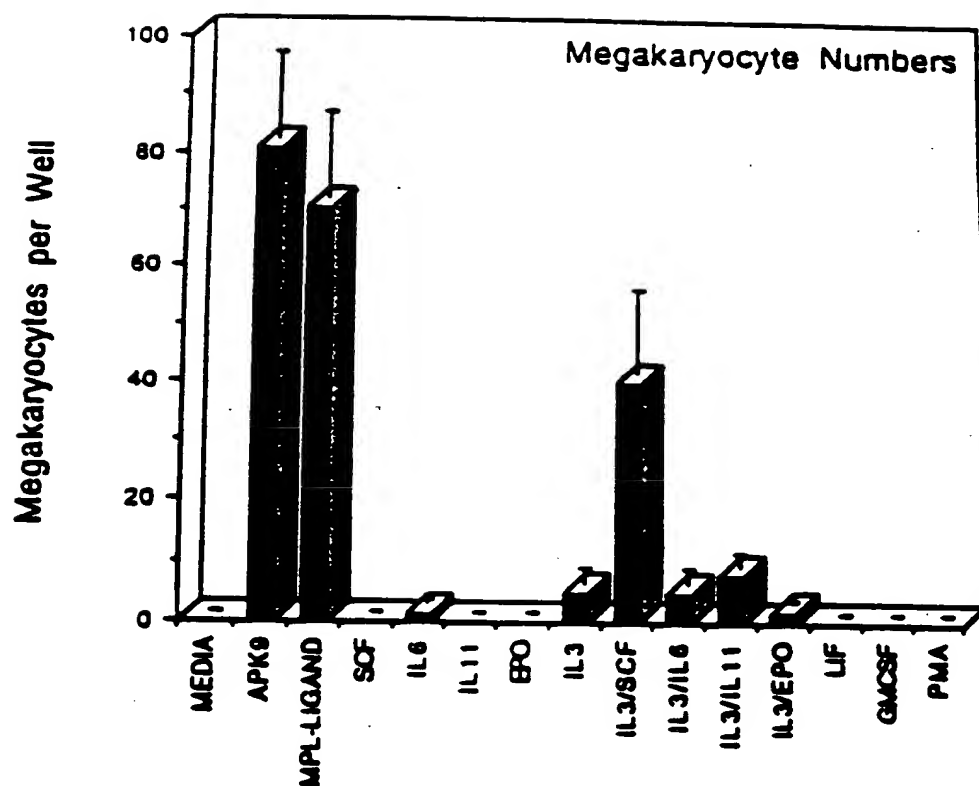


Figure 7

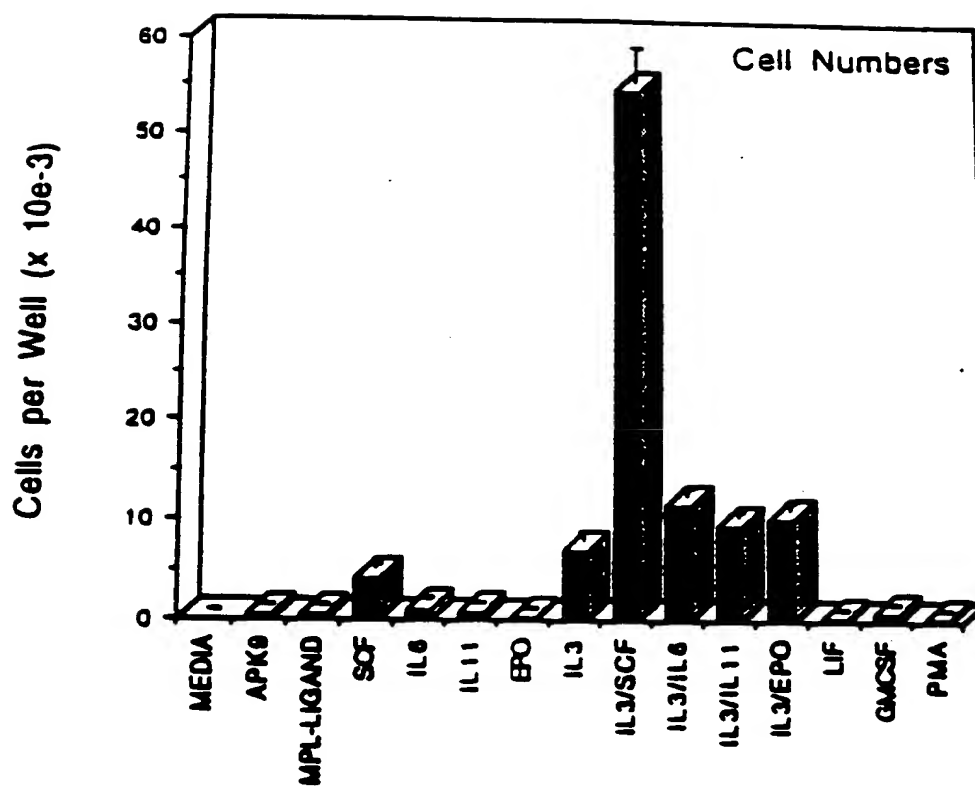


Figure 8

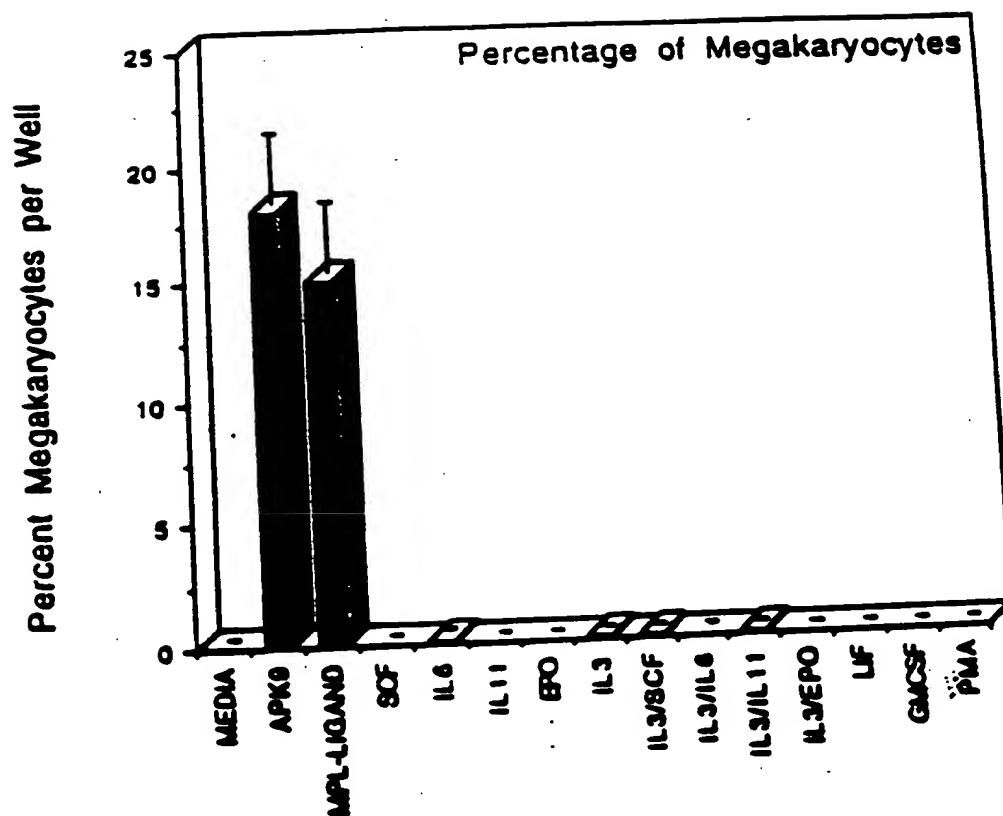


Figure 9

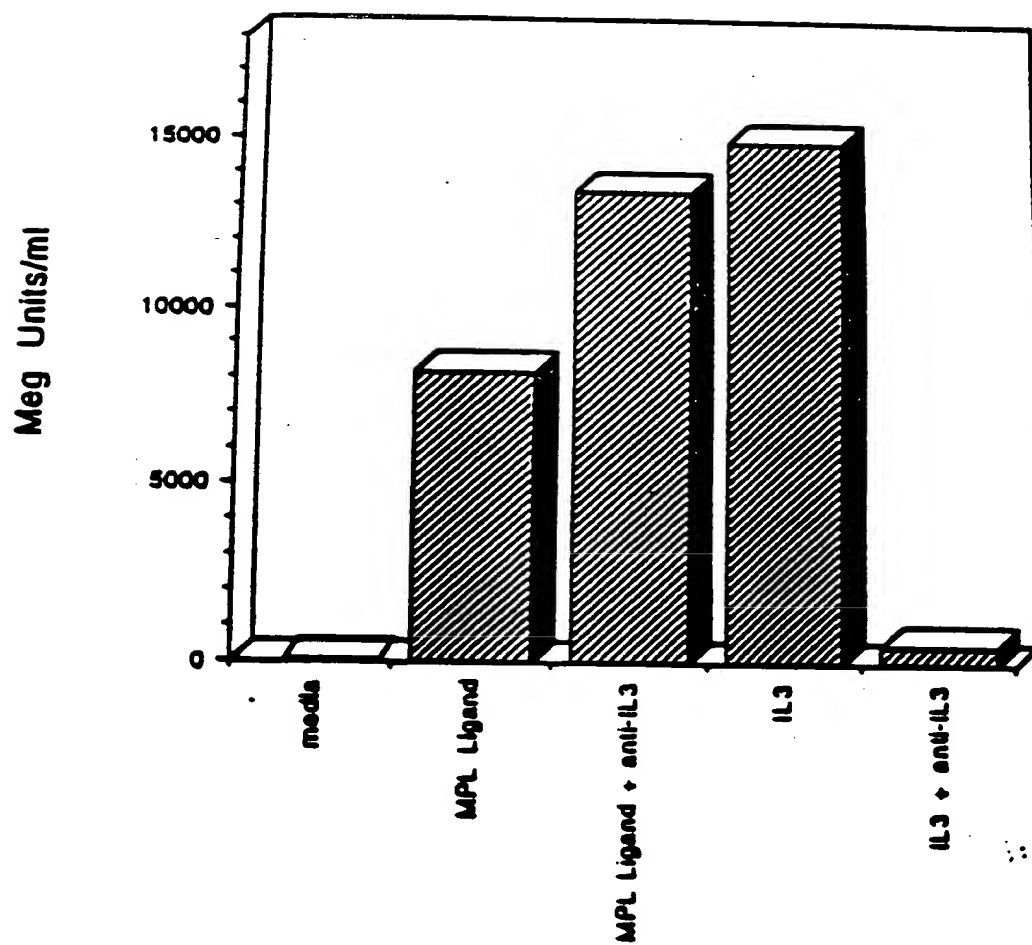


Figure 10

1	CAGGAGCCACGCGAGGCAAGACACCGCGGAGATCGAGCTGACTGAATTGCTCTC	59
1	MetGlnLeuThrGlnLeuLeuLeu	8
60	GAGGTCATGCTTCTCTAACTCAAGCTTAAGCTTCTAGCGCGGCTCTCTCTCTCT	119
9	ValValMetLeuLeuLeuThrAlaArgLeuThrLeuSerSer9 to Ala2 to 9 to AlaCys	28
120	GACCTCGAGTCTCTAGTAAACTGCTTCTGACTCCCACTGCTTTCACAGCAGACTGAGC	179
29	AspLeuArgValLeuSerLysLeuLeuArgAspSerHisValLeuHisSerArgLeuSer	48
180	CAGTCCCCAGGGTTCACCTTTCCCTACACCTGTCTCTCTCTCTCTCTCTCTCTCT	239
49	GlnCys9 to GlnValHis9 to Leu9 to Thr9 to ValLeuLeu9 to AlaValAspPheSer	68
240	TTGGGAGATCGAAACCCAGTGGAGGAGCCAGGCGACGGACATTCTGGGAGGCTG	299
69	LeuGlyGlnTrpLysThrGlnMetGluGluThrLysAlaGlnAspIleLeuGlyAlaVal	88
300	ACCTTCTCTCTGAGGGAGTGTATGCGAGGACCGGACAACTGGGAGCCACTTCTCTCA	359
89	ThrLeuLeuGluGlyValMetAlaAlaArgGlyGlnLeuGly9 to ThrCysLeuSer	108
360	TGCTCTCTGGGACAGCTTCTCGACAGTCTCTCTCTCTCTCTCTCTCTCTCTCTCTCT	419
109	SerLeuLeuGlyGlnLeuSerGlyGlnValArgLeuLeuLeuGlyAlaLeuGlnSerLeu	128
420	CTTGGAAACCCAGCTTCTCTCAGGGGAGGACCAAGCTCAGAGGATCCCAATGCCATC	479
129	LeuGlyThrGlnLeu9 to 9 to GlnGlyArgThrThrAlaHisLysAsp9 to AsnAlaIle	148
480	TTCTGAGCTTCCAACTCTCTCTGAGGAAAGGTGCGTTTCTCTGATCTTGTAGGAGG	539
149	PheLeuSerPheGlnHisLeuLeuArgGlyLysValArgPheLeuMetLeuValGlyGly	168
540	TCCACCTCTCTCTGAGGCGGCGCGCCACCCACCACTGTCTCTCTCTCTCTCTCTCTCT	599
169	SerThrLeuCysValArgArgAla2 to 9 to ThrThrAlaVal9 to SerArgThrSerLeu	188
600	GTCTTCACTGACCTGCTCCCAACAGGACTTCTGATTTGTTGGAGCAAACTTCACT	659
189	ValLeuThrLeuAsnGluLeu9 to AsnArgThrSerGlyLeuLeuGluThrAsnPheThr	208
660	GCTCAGCCAGACTCTGCTCTCTGCGCTTCTGAGTGGCAGCGGATTCAGAGCCAG	719
209	AlaSerAlaArgThrThrGlySerGlyLeuLeuLysTrpGlnGlnGlyPheArgAlaLys	228
720	ATTCTGCTCTCTCTGACCAACCTCCAGTCTCTCTGACCAATCCCGGATCTCTGAC	779
229	Ile9 to GlyLeuLeuAsnGlnThrSerArgSerLeuAspGlnIle9 to GlyTyrLeuAsn	248
780	AGGATACAGACTCTTCTGATCGACTCTCTGACTCTTCTCTGACCTCTACCGAGGCT	839
249	ArgIleHisGluLeuLeuAsnGlyThrArgGlyLeuPhe9 to Gly9 to SerArgArgThr	268
840	CTAGGAGCCCGGACATTCTCTAGGACATCGACAGGCTCTCTCTCTCTCTCTCTCTCT	899
269	LeuGlyAla9 to AspIleSerSerGlyThrSerAspThrGlySerLeu9 to 9 to AsnLeu	288
900	CAGCTGAGGCT	959
289	Gln9 to GlyTyrSer9 to Ser9 to ThrHis9 to 9 to ThrGlyGlnTyrThrLeuPhe9 to	308
960	CTTCT	1019
309	Leu9 to 9 to ThrLeu9 to Thr9 to ValValGlnLeuHis9 to LeuLeu9 to Asp9 to Ser	328
1020	GCTCCAGCCCGGACCTTCT	1079
329	Ala9 to Thr9 to Thr9 to ThrSer9 to LeuLeuAsnThrSerTyrThrHisSerGlnAsn	348
1080	CTGCTCTAGGAGGCTAGGTTCTCTGACCTCCCGACATCGCAATTGCTCTCTCTCTCT	1139
349	LeuSerGlnGluGlyAsn	353
1140	CT	1199
1200	AAACCCAAAGCTCTCTAAAGGCTACAGGCTGAAAGGCTATTTTCTCTCTCTCTCT	1259
1260	ACATATTAAGCTTCTGAGCTATTTTCTAGCTATCGCAACTCTCTCTCTCTCTCTCT	1319
1320	AGCTCTTTCT	1342

Figure 11

1	AGGGAGCCACGCCGACAGACACCGCGGCAGATGGAGTGAATTGCTCTCTGTG	60
	MetGluLeuThrGluLeuLeuVal	9
61	GTCATGCTTTCTCTAACTGCAAGGCTAACGCTGTCCAGCCCGGCTCTCTCTGTGTGAC	120
10	ValMetLeuLeuLeuThrAlaArgLeuThrLeuSerSerProAlaProAlaCysAsp	29
121	CTCCAGTGCTCAGTAACACTGCTTCTGTACTCCCATGTCTTACAGCAGACTGAGCCAG	180
30	LeuArgValLeuSerLysLeuLeuArgAspSerHisValLeuHisSerArgLeuSerGln	49
181	TGCCAGAGGTTTACCCCTTTGCTACACCTGTCTCTGTCTCTGTGTGACTTTAGCTTG	240
50	CysProGluValHisProLeuProThrProValLeuLeuProAlaValAspPheSerLeu	69
241	GGAGAATGGAAAACCAGATGGAGGAGACCAAGCCACAGGACATTCTGGAGCGGTGACC	300
70	GlyGluTrpLysThrGlnMetGluGluThrLysAlaGlnAspIleLeuGlyAlaValThr	89
301	CTTCTGCTGGAGGGAGTGATGGCAGCACGGGGCAACTGGGACCCACTTCTCTCTATCC	360
90	LeuLeuLeuGluGlyValMetAlaAlaArgGlyGlnLeuGlyProThrCysLeuSerSer	109
361	CTCTGGGGGAGCTTTCTGGACAGTCCGTCTCTCTCTGGGGCCCCGACAGAGCTCTTT	420
110	LeuLeuGlyGlnLeuSerGlyGlnValArgLeuLeuLeuGlyAlaLeuGlnSerLeuLeu	129
421	GGAACCCAGCTTCTCTCCACAGGGCAGGACCCACAGCTCACAAGATCCCATCTTTC	480
130	GlyThrGlnLeuProProGlnGlyArgThrThrAlaHisLysAspProAsnAlaIlePhe	149
481	CTGAGCTTCCACACTCTCTCCAGGAAGGACTTCTGAGTTGTTGGAGCAAAGTTCC	540
150	LeuSerPheGlnHisLeuLeuArgGlyLysAspPheTrpIleValGlyAspLysLeuHis	169
541	TGCTCAGCCAGAACTACTGGCTCTGGCTTCTGAAGTGGCAGCAGGATTCCAGCCAA	600
170	CysLeuSerGlnAsnTyrTrpLeuTrpAlaSerGluValAlaAlaGlyIleGlnSerGln	189
601	GATTCTGGTCTGCTGAACAAACTCCAGGTCCCTGGACCAATCCCCGATACTGAA	660
190	AspSerTrpSerAlaGluProAsnLeuGlnValProGlyProAsnProArgIleProGlu	209
661	CAGATACAGGACTCTTGATGGAACTCTGCACTTTTCTGGACCCCTACGGCAGGAC	720
210	GlnAspThrArgThrLeuGluTrpLeuSerTrpThrLeuSerTrpThrLeuThrGlnAsp	229
721	OCTAGGAGCCCCGACATTCTCTCAGGACATCAGACACAGGCTCTCTCCACCCAACT	780
230	ProArgSerProGlyHisPheLeuArgAsnIleArgHisArgLeuProAlaThrGlnPro	249
781	CCAGCTGGAGTTCTCTCTTCCCAACCATCTCTCTACTGGACATATAGCTCTTCC	840
250	ProAlaTrpIlePheSerPheProAsnProSerSerTyrTrpThrValTyrAlaLeuPro	269
841	SETTCCGCCGCTTCCCAACCTCTGTGTCCAGTCCACCCCTCTCTCTGTACCCCTTC	900
270	SerSerThrHisLeuAlaHisProCysGlyProAlaProProProAlaSerEnd	289
901	TCTCCCAAGCCGACCTCTACAGCCCTCTTCTAAGCATCTCTACACCCACTCCAGAA	960
961	TCTCTCTCAGAGGGAAGTTCTCTGACACTCCCCACATCAGCAATTCTCTGTGTGAC	1020
1021	GCTCTCTTCTCTCAGGCGGCGCTGGAGCAACTGGACAGATTCTCTACTTTCTCT	1080
1081	GAACCCAAAGCTTCTAAGGCTGACACAGGACTGAAGGGAATCTTTTCTCT	1140
1141	TACTATTAAGCTTCTGAGTA	1164

Figure 12

Figure 13A

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murine 1 MELTDLLLAAMLLAVARLTLSPPVAPACDPRLNKLRLROSHLLHSRLSQC 50
      |||:||||.|||.|||||||.|||:|.||||:|||||
human 1 MELTELLLVVMLLLTARLTLSPPAPACDLRVLSKLLRDSHVLSHSRLSQC 50

      51 PDVDP L S I P V L L P A V D F S L G E W K T Q T E Q S K A Q D I L G A V S I L L E G V M A A R G 100
      |:|.|||.|||.|||||||.|||||||.|||||||.|||||
      51 P E V H P L P T P V L L P A V D F S L G E W K T Q M E E T K A Q D I L G A V T I L L E G V M A A R G 100

      101 Q L E P S C L S S I L G Q L S G Q V R L L L G A L Q G L L G T Q L P L Q G R T T A H K D P M A I F L 150
      |||.|||||||.|||||||.|||||||.|||||||.|||||
      101 Q L G P T C L S S I L G Q L S G Q V R L L L G A L Q S L L G T Q L P P Q G R T T A H K D P M A I F L 150

      151 S L Q Q L L R G K V R F L L L V E G P T L C V R R T L P T T A V P S S T S Q L L T L N K P M R T S 200
      |:|:|||||||.|||.|||||.|||||||.|||.|||.|||.
      151 S F Q H L L R G K V R F L M L V G G S T L C V R R A P P T T A V P S R T S L V L T L N K L P M R T S 200

      201 G L L E T N F S V T A R T A G P G L L S R L Q G F R V K I T P G Q L N Q T S R S P V Q I S G Y L M R 250
      ||||||.|||.|||.|||.|||.|||.|||.|||.|||.|||.
      201 G L L E T N T A S A R T T G S G L L K W Q Q G F R A K I . P G L L N Q T S R S L D Q I P G Y L M R 249

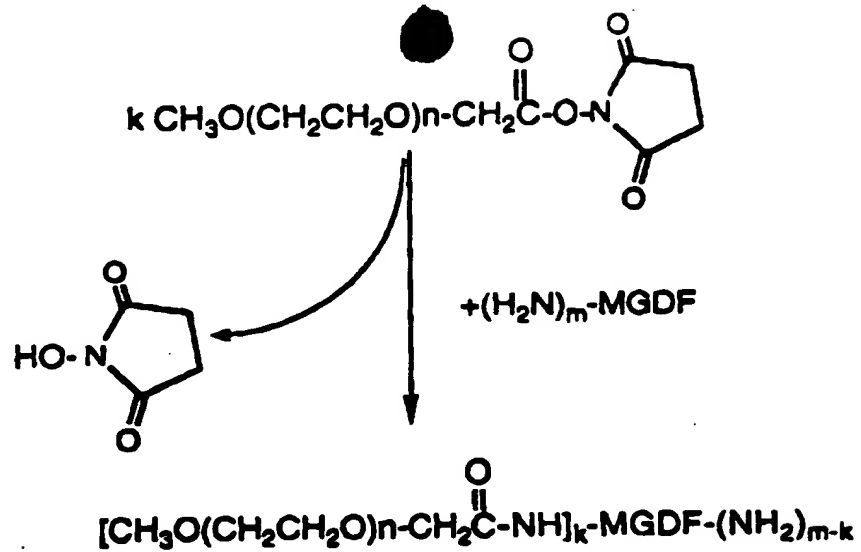
      251 T H G P V N G T R G L F A G T S L Q T L E A S D I S P G A F N K G S L A F N L Q G G L P P S P S L A 300
      .|:|.|||:|||||.|||.|||.|||||.|||.|||.|||.
      250 I H E L L N G T R G L F P G P S R R T L G A P D I S S G T S D T G S L P P N L Q P G Y S P S P T H P 299

      301 P D G H . T P F P P S P A L P T T H G S P P Q L H P L F D P S T T H P N S T A P H P V M K Y H P 349
      |.:| | | | .|.|||.|||.|||||||.|||.|||.|||.
      300 P T G Q Y T L F P L P T L P T . . . P V V Q L H P L L P D P S A P T P T P T S P L L N T S Y T H S 346

      350 R N L S Q E T 356
      .|||||.
      347 Q N L S Q E G 353

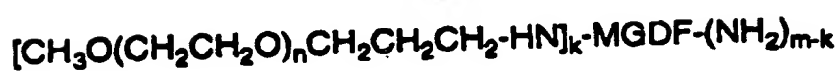
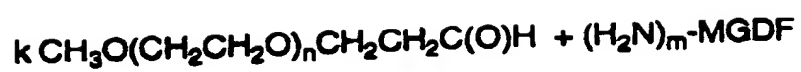
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Figure 13B



k , m and n are the same as defined in Figure 15.

Figure 14



- k** - number of PEG molecules reacted with a molecule of MGDF;
- n** - degree of polymerization of PEG used in the reaction; e.g. $n=2000$ for PEG of MW=100 kD; $n=40$ for PEG of MW=2 kD.
- m** - total number of primary amino groups per MGDF molecule.

Figure 15

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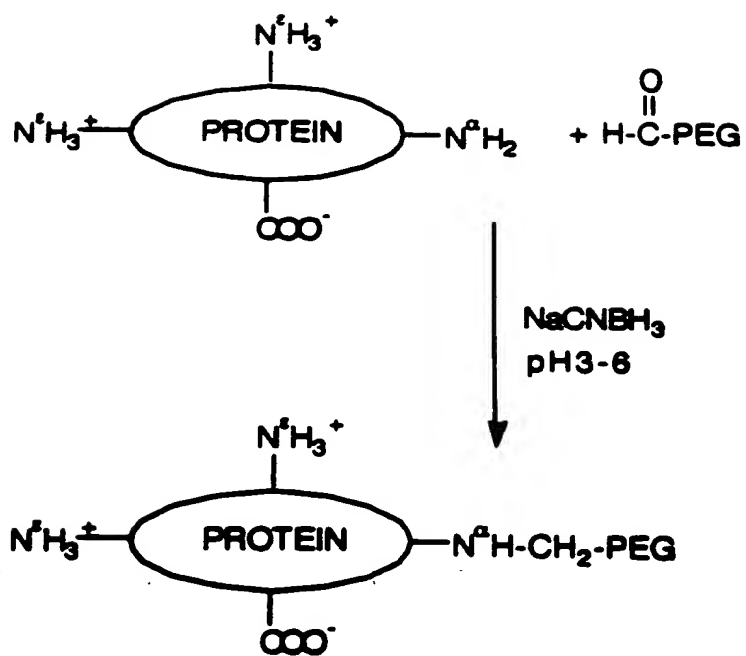


Figure 16

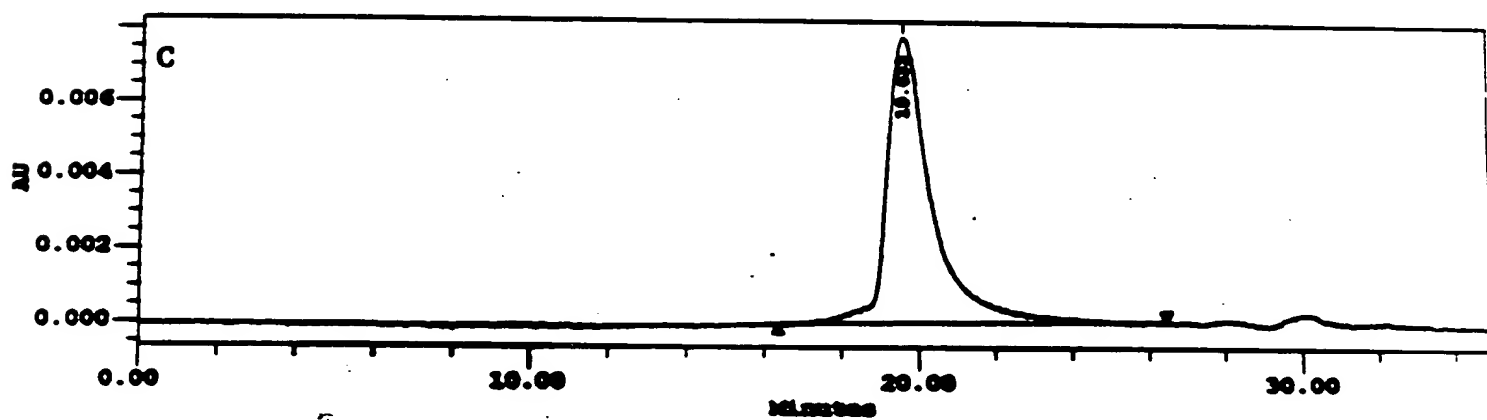
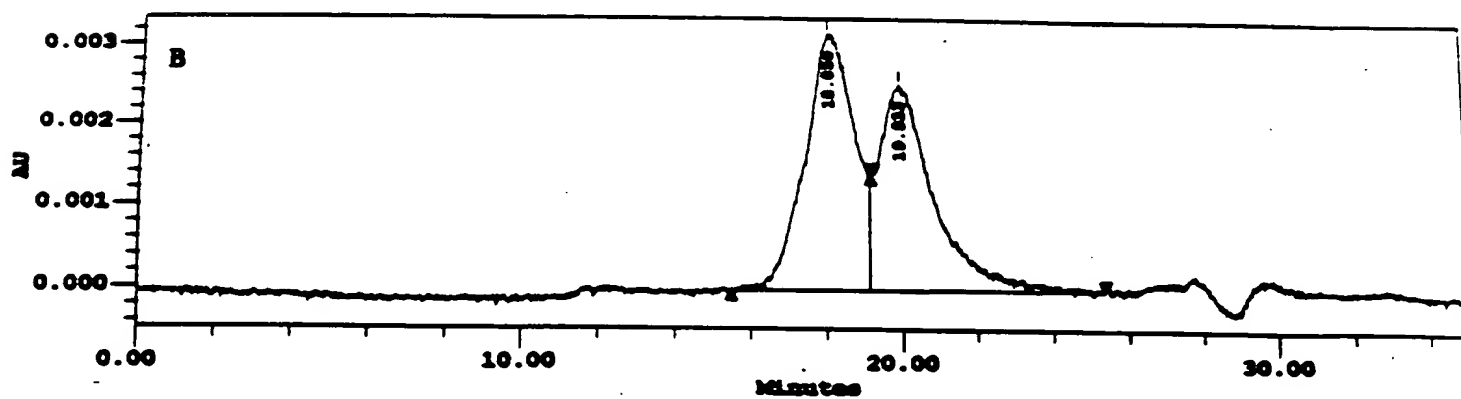
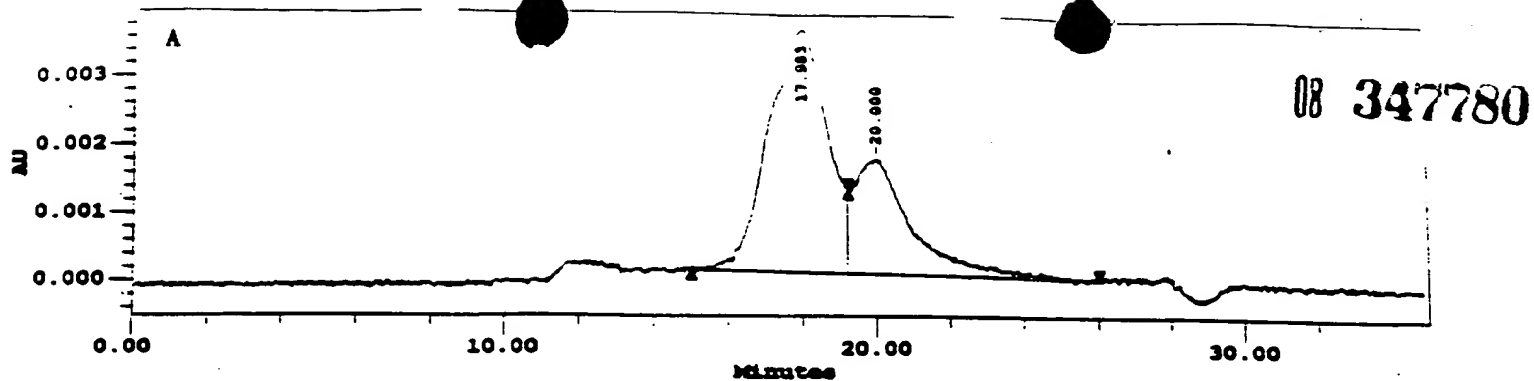


Figure 17

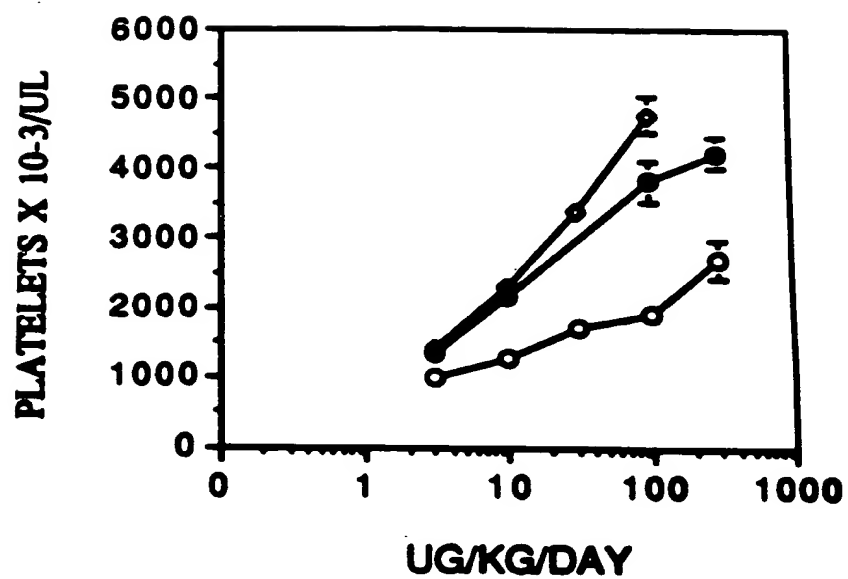


Figure 18

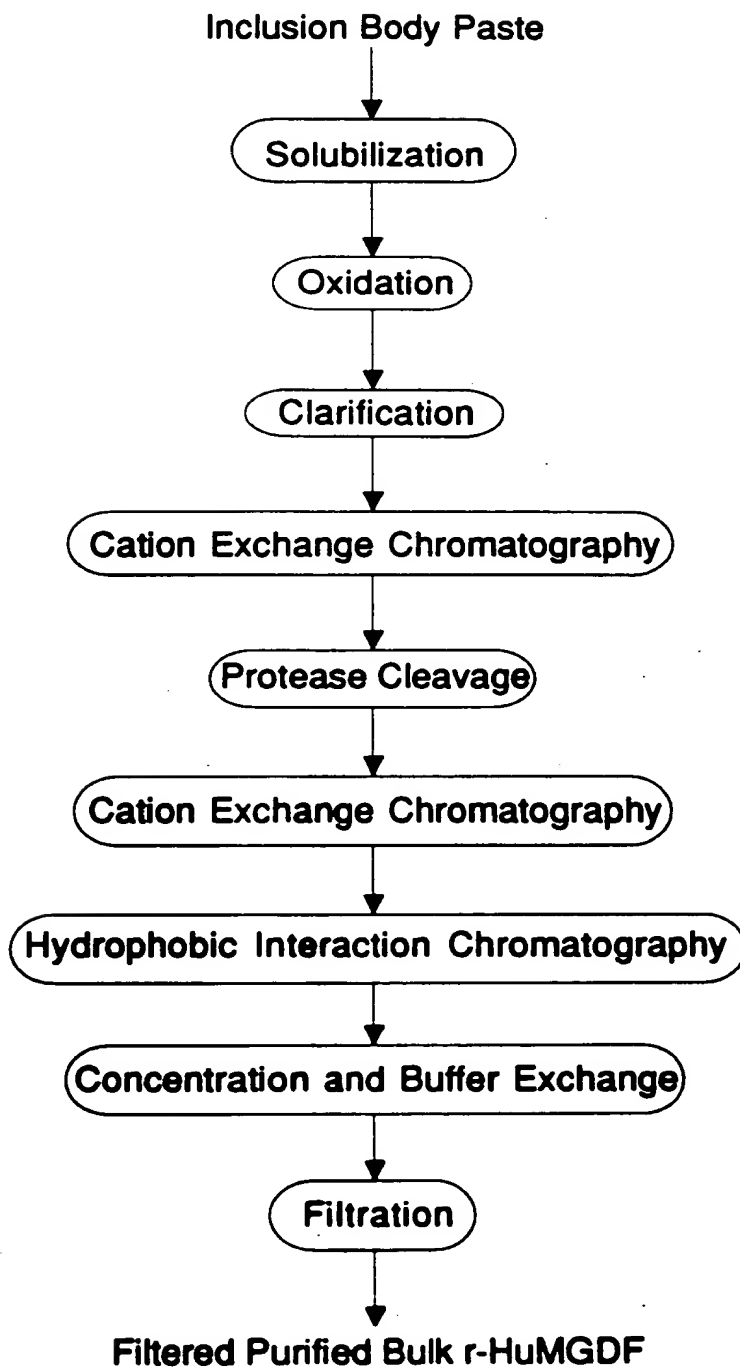
Purification Flow Chart for r-HuMGDF

Figure 19

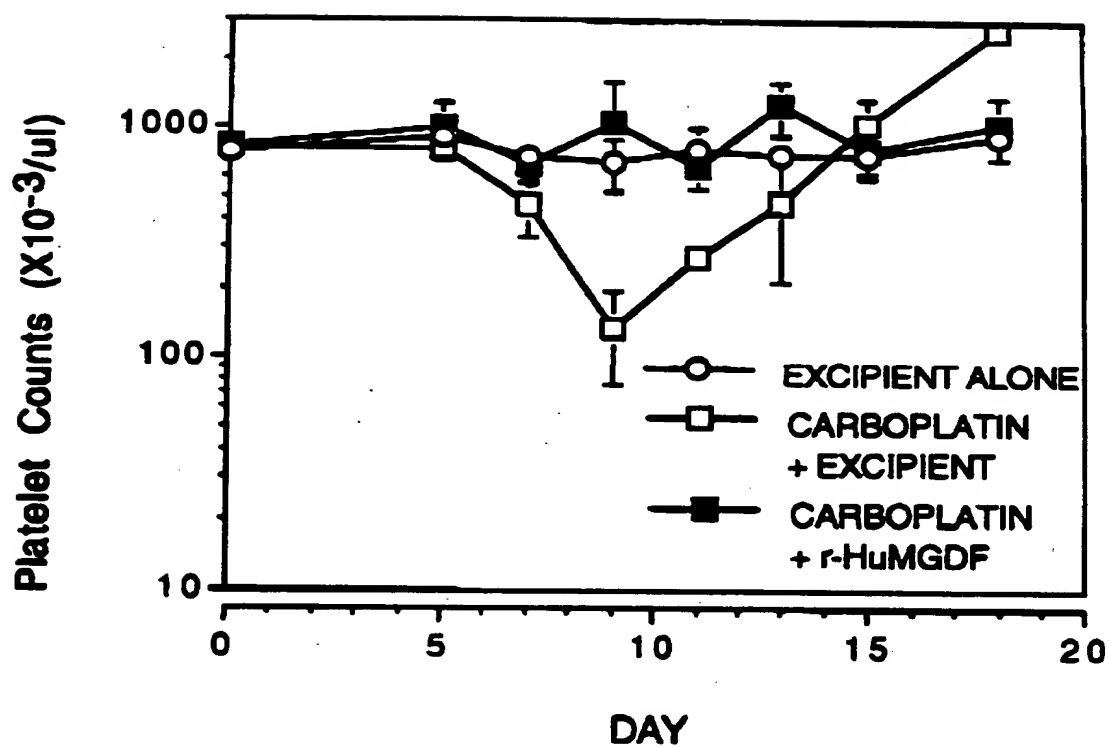


Figure 20

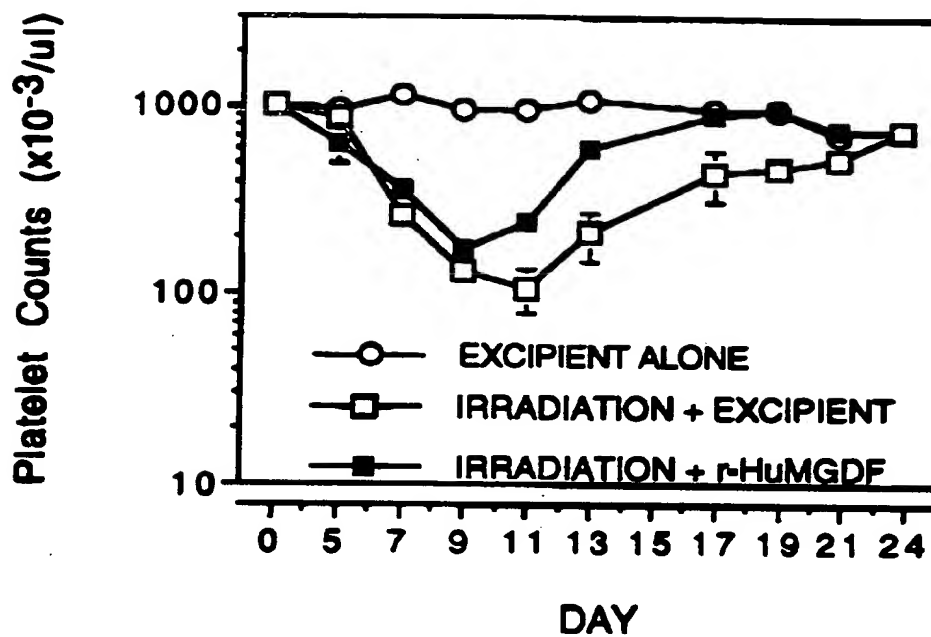


Figure 21

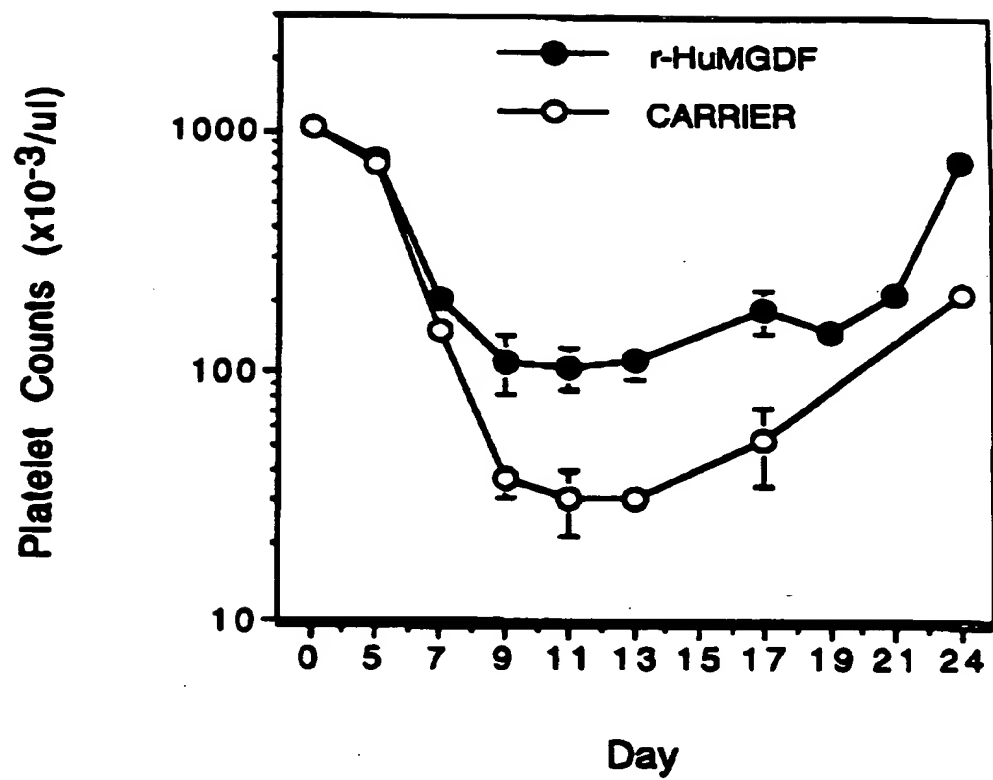


Figure 22

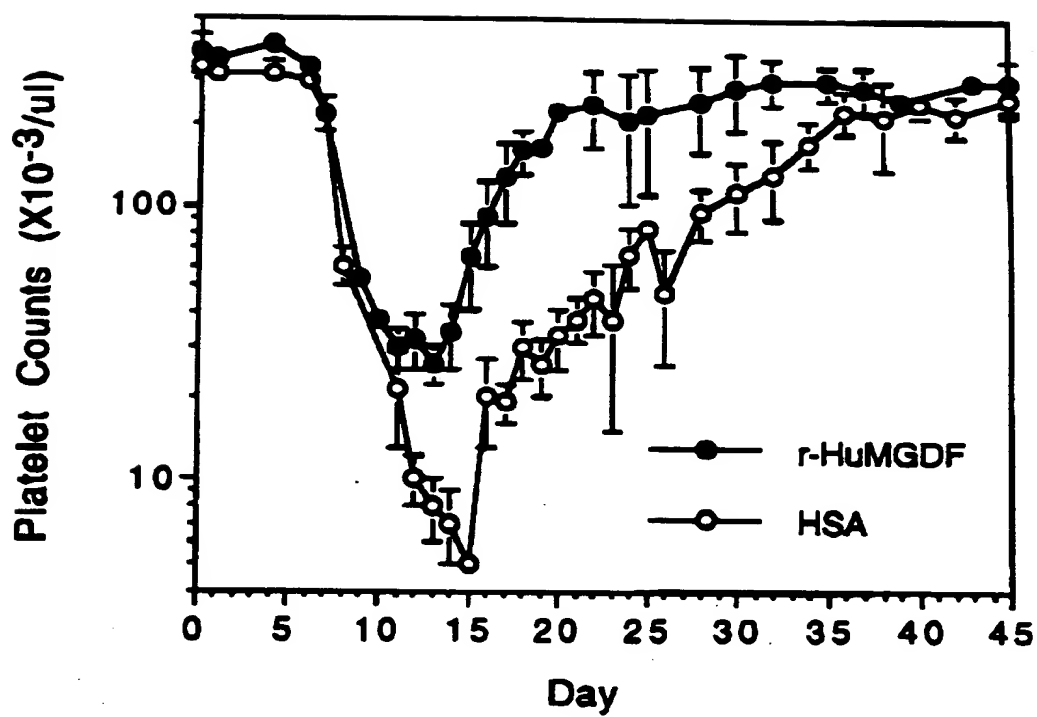


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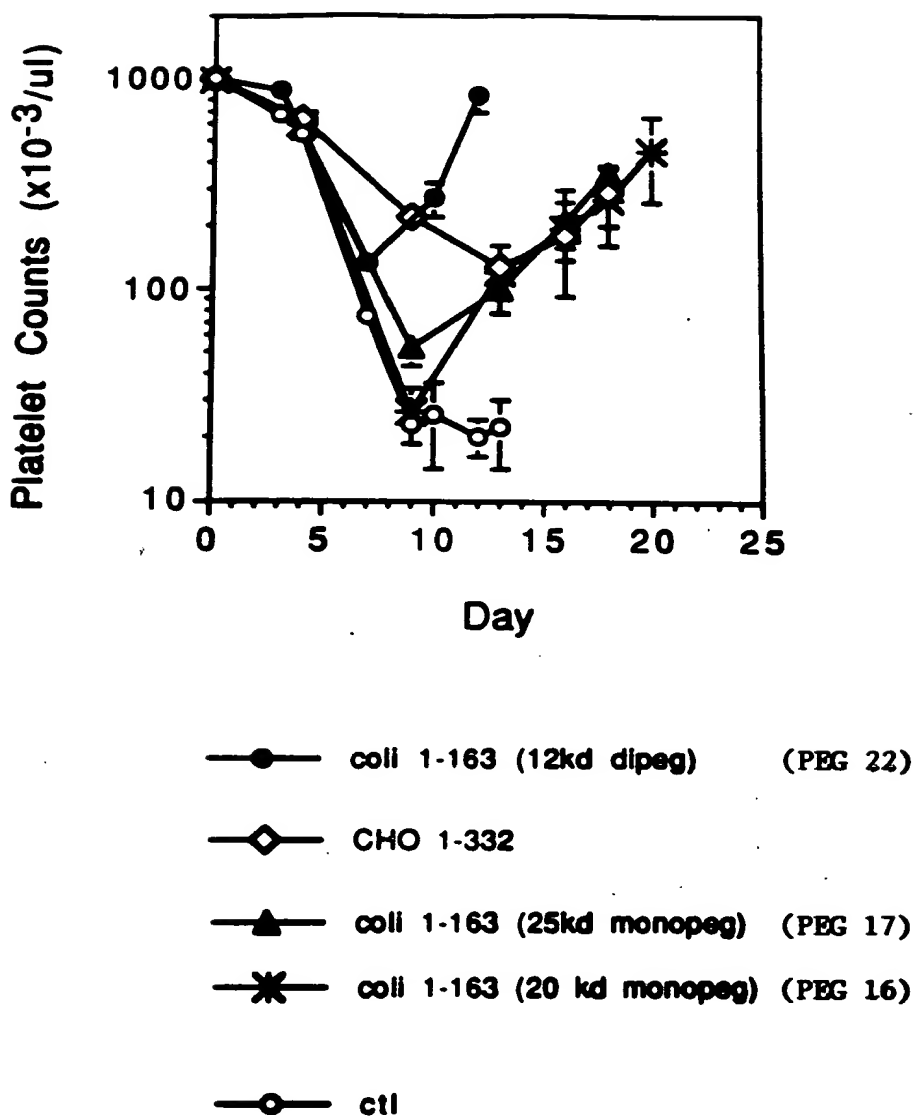


Figure 24

r-HuMGDF (1-163) Translation

ATG	AAA	AGT	CCT	GCA	CCA	CCT	GCA	TGT	GAT	TTA	CGG	GTC	CTG
MET	LYS	SER	PRO	ALA	PRO	PRO	ALA	CYS	ASP	LEU	ARG	VAL	LEU
TCT	AAA	CTG	CTG	CGC	GAC	TCT	CAC	GTG	CTG	CAC	TCT	CGT	CTG
SER	LYS	LEU	LEU	ARG	ASP	SER	HIS	VAL	LEU	HIS	SER	ARG	LEU
TCC	CAG	TGC	CCG	GAA	GTT	CAC	CCG	CTG	CCG	ACC	CCG	GTT	CTG
SER	GLN	CYS	PRO	GLU	VAL	HIS	PRO	LEU	PRO	THR	PRO	VAL	LEU
CTT	CCG	GCT	GTC	GAC	TTC	TCC	CTG	GGT	GAA	TGG	AAA	ACC	CAG
LEU	PRO	ALA	VAL	ASP	PHE	SER	LEU	GLY	GLU	TRP	LYS	THR	GLN
ATG	GAA	GAG	ACC	AAA	GCT	CAG	GAC	ATC	CTG	GGT	GCA	GTA	ACT
MET	ALA	ALA	ARG	LYS	ALA	GLN	ASP	ILE	LEU	GLY	ALA	VAL	THR
CTG	CTT	CTG	GAA	GGC	GTT	ATG	GCT	GCA	CGT	GGC	CAG	CTT	GGC
LEU	LEU	LEU	GLU	GLY	VAL	MET	ALA	ALA	ARG	GLY	GLN	LEU	GLY
CCG	ACC	TGC	CTG	TCT	TCC	CTG	CTT	GGC	CAG	CTG	TCT	GGC	CAG
PRO	THR	CYS	LEU	SER	SER	LEU	LEU	GLY	GLN	LEU	SER	GLY	GLN
GTT	CGT	CTG	CTG	CTC	GGC	GCT	CTG	CAG	TCT	CTG	CTT	GGC	ACC
VAL	ARG	LEU	LEU	LEU	GLY	ALA	LEU	GLN	SER	LEU	LEU	GLY	THR
CAG	CTG	CCG	CCA	CAG	GGC	CGT	ACC	ACT	GCT	CAC	AAG	GAT	CCG
GLN	LEU	PRO	PRO	GLN	GLY	ARG	THR	THR	ALA	HIS	LYS	ASP	PRO
AAC	GCT	ATC	TTC	CTG	TCT	TTC	CAG	CAC	CTG	CTG	CGT	GGC	AAA
ASN	ALA	ILE	PHE	LEU	SER	PHE	GLN	HIS	LEU	LEU	ARG	GLY	LYS
GTT	CGT	TTC	CTG	ATG	CTG	GTT	GGC	GGT	TCT	ACC	CTG	TGC	GTT
VAL	ARG	PHE	LEU	MET	LEU	VAL	GLY	GLY	SER	THR	LEU	CYS	VAL
CGT	CGG	GCG	CCG	CCA	ACC	ACT	GCT	GTT	CCG	TCT	TAA		
ARG	ARG	ALA	PRO	PRO	THR	THR	ALA	VAL	PRO	SER	STOP		

Figure 25